For Reference

NOT TO BE TAKEN FROM THIS ROOM

For Reference

NOT TO BE TAKEN FROM THIS ROOM

Ex libris
universitatis
albertaensis

QUAECUMQUE VERA

The University of Albert
Printing Department
Edmonton, Alberta

Digitized by the Internet Archive
in 2023 with funding from
University of Alberta Library

https://archive.org/details/Walker1970_0

THE UNIVERSITY OF ALBERTA

GENETIC MAPPING OF MALE STERILITY MUTANTS IN *NEUROSPORA CRASSA*

by

© David G. Walker

A THESIS
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE
OF MASTER OF SCIENCE

DEPARTMENT OF GENETICS

EDMONTON, ALBERTA

SPRING, 1970

137

UNIVERSITY OF ALBERTA

The undersigned certify that they have read and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "Genetic Mapping of Male Sterility Mutants in *Neurospora Crassa*" submitted by David G. Walker, in partial fulfillment of the requirements for the degree of Master of Science.

Abstract

Nine male sterile (*ster*) mutants previously described by Vigfusson, were used in two-, three-, and four-point crosses with known marker strains of *Neurospora crassa* in order to determine their relationship with established linkage groups. The male sterility gene as present in mutant 8455 *A*, *leu*-3, *ster* was found to be located in Linkage Group VII on the basis of the observed recombination frequency between the genes *ster* and *nic*-3.

The recombination data for the mutants:

10178 *A*, *leu*-3, *ster*
10710 *A*, *leu*-3, *ster*
10710-1 *A*, *leu*-3, *ster*
10299 *A*, *leu*-3, *ster*
7232 *A*, *leu*-3, *ster*
5538 *A*, *pan*-1, *ster*
16009 *A*, *pan*-1, *ster*

were found to be indicative for linkage with markers of Linkage Group I.

Crosses involving the male sterile strain 8553 *A*, *leu*-3, *ster* yielded results which could be interpreted as either due to quasi linkage between *ster* and *mt* (mating type, Linkage Group I) or to abnormally high crossover frequencies in the intercept *leu*-3 to *mt* (Linkage Group I) resulting from the presence of the gene *ster* in that region.

Several crosses involving pantothenic acid requiring strains in matings with non-pantothenic acid requiring strains, did not yield the expected pantothenic acid requiring progenies when the gene for male sterility (*ster*) was also involved in the cross. The possibility that selection for *pan*-1^+ segregants underlies the non-Mendelian segregation for this character, has been discussed.

Acknowledgements

I wish to thank Dr. J. Weijer for his guidance during the course of this study. I would also like to thank Dr. N.V. Vigfusson who provided the mutants and much invaluable assistance in handling them. Technical assistance from Mr. P. Ager is also acknowledged. Thanks also to the whole staff of the Genetics Department for their support.

This study was supported in part by a grant from the National Research Council of Canada to Dr. Weijer.

TABLE OF CONTENTS

Page

INTRODUCTION AND LITERATURE REVIEW........................ 1

MATERIALS AND METHODS.................................... 6

1. Strains.. 6

2. Media.. 6

3. Methods.. 9

RESULTS.. 12

(i) Recombination data for mutant 8455................. 12

(ii) Recombination data for mutant 8553................ 17

(iii) Recombination data for mutant 10718.............. 18

(iv) Recombination data for mutant 10710............... 21

(v) Recombination data for mutant 10710-1.............. 23

(vi) Recombination data for mutant 10299............... 25

(vii) Recombination data for mutant 7232............... 27

(viii) Recombination data for mutant 5538.............. 28

(ix) Recombination data for mutant 16009............... 31

DISCUSSION AND CONCLUSIONS............................... 33

APPENDIX... 46

BIBLIOGRAPHY... 52

LIST OF TABLES

Page

Table I. Description of stock cultures used.................... 7

Table II. Description of male sterile mutant cultures used...... 8

Table III. Recombination data for mutant 8455................... 13, 14

Table IV. Recombination data for mutant 8553................... 16

Table V. Recombination data for mutant 10718................... 19

Table VI. Recombination data for mutant 10710................... 20

Table VII. Recombination data for mutant 10710-1................ 22

Table VIII. Recombination data for mutant 10299.................. 24

Table IX. Recombination data for mutant 7232................... 26

Table X. Recombination data for mutant 5538................... 29

Table XI. Recombination data for mutant 16009.................. 30

Table XII. Analysis of the recombination data from the cross: 8553 A, leu-3, ster x B 110 a, fr..................... 36, 37

Table XIII. Analysis of the recombination data from the cross: 8553 A, leu-3, ster x R 2360 a, cr................... 38, 39

Table XIV. Analysis of the recombination data from the cross: 8553 A, leu-3, ster x 56501 a, thi-1................. 40, 41

Introduction

In fungi, the formation of sexual organs is genetically determined. Incompatibility factors on the other hand, determine the mating potential of each individual. These two systems help to promote genetic recombination through the prevention of self-fertilization.

Heterothallic fungi, and more specifically the Ascomycete *Neurospora crassa*, incorporate an incompatibility system consisting of one locus with two alleles (*a* *vs.* *A*). This pair of alleles segregates at meiosis to produce 4 ascospores of mating type *A* and 4 ascospores of mating type *a*. Ascosporogenesis results from matings in which mating type *A* functions as a protoperithecial (or recipient) parent and mating type *a* as the spermatial (or fertilizing) parent or the converse. The female organ is fully differentiated according to its particular function as recipient parent whereas spermatial elements such as conidia, hyphae and trichogyne are not specific in morphology or function.

Since sexuality in fungi not only involves a simple act of karyogamy, but consists of a sequence of events starting at hyphal attraction and terminating with the production of ascospores, the genetic determination of such a sequela is extremely complex. Whitehouse (1949) and Lewis (1954), therefore, suggested that a simple but compound mating type locus constitutes the determining genetic region for all these steps of sexuality. Burnett (1956) on the other hand postulates the possibility that the mating type factors are "super-genes" which act as "switch" genes in controlling other genes which regulate the mating action.

Although complexity of the locus is indicated, the locus nevertheless appears to be very stable. Few reports of variants of the mating type locus exist. Lindegren (1934) found 4 bisexual and 4 akaryotic spores in one ascus of *Neurospora crassa*. The 4 bisexual spores were self-fertile. Olive (1958) states that St. Lawrence obtained several single spored isolates of *N. crassa* which were not self-fertile but could cross with either mating type *A* or *a*. These findings were taken to indicate that an incompatibility block to self-fertility may exist. Moureau and Moruzi (1932) in *N. sitophila* observed a similar case in which 4 spored asci were found to be homothallic in their mating type reaction. Newmeyer (1965) crossed an *arg*-1 strain to the wild type and observed that about 1/4 of the viable ascospores initially yielded colonies which formed dark pigment on complete medium. These "Dark Agar" colonies eventually "escaped" the inhibition and grew like the wild type. "Dark Agar" colonies could cross with either mating type *A* or *a*; "escaped" colonies contained conidia with a mixture of *A* and *a* mating type determining nuclei. On the basis of the 6 viable to 2 non-viable abortion pattern Newmeyer postulates that a pericentric inversion could yield a viable duplication-deficiency class and a non-viable duplication-deficiency class among the segregants. Heterozygosity of the mating type locus accounted for the initial bisexual "Dark Agar" colonies and the "escaped" colonies formed when homozygous *A* and *a* nuclei resulted from mitotic crossing over.

Weijer and Yang (1966) reported a self-sterile bisexual spore in an ascus along with 3 normal mating type *A* and 4 normal mating type *a*. According to these authors, the results are indicative for a compound nature of the mating type locus.

For long it has been known, that prevention of incompatible matings between isolates of the same mating type is exercised at several different steps in the sexual cycle. For instance Backus (1939) reported, that there is no evidence for fusion between conidia and trichogyne of the same mating type in *N. sitophila*. Plasmogamy, on the other hand, has been found to occur between identical mating types of *Ascobolus stercorarius* although no karyogamy was observed (Bistus and Raper, 1963). From the literature it is evident that the control that excludes incompatible matings probably occurs in different fungi at different stages of the sexual process.

Another approach to inquire into the sexual development in fungi concerns a study of the secondary physiological control mechanism (Raper, 1960). This control system governs the sequential progression of events from trichogyne attraction (if any) to plasmogamy, karyogamy and meiosis. Although this step by step sequence is under genetic control it is doubtful as to whether such a control is exercised by the actual mating type locus as part of the incompatibility regulation.

Dodge (1935) described three distinct stages of perithecial development and maturation in *Neurospora sitophila* and *N. tetrosperma*:

a) The formation of the ascogonium together with the development of surrounding hyphal tissue to form the incipient ascocarp;

b) the bringing together of nuclei of opposite mating type, differentiation of wall tissue, development of ascogenous hyphae, and nuclear fusion; and,

c) the reduction division, spore delimination and spore maturation.

The most extensive study of gene control of sequential steps in the sexual cycle have been made in the homothallic, hermaphroditic fungi, Glomerella cinqulata. Wheeler and McGahen (1952) and Wheeler (1954) found nine genes affecting sexual reproduction. These genes were found to control the developmental sequela starting with the ability to produce the occurence of perithecia and ending with the ability to produce viable ascospores. On the basis of developmental blocks four major steps were indicated to occur in the sexual cycle: perithecial initiation, plasmogamy, karyogamy and meiosis. These mutants were self-sterile though cross-fertile indicating that complementation of function occurs in cross matings. Their novel work still remains the most extensive study concerning developmental steps and their progression in the sexual process of fungi.

Far less is known of blocks occuring in the sexual cycle of heterothallic Ascomycetes. Westergaard and Hirsh (1954) reported two abnormalities concerning the lack of protoperithecia and abnormal protoperithecia. Both abnormalities were found to be due to two different genes. Also in N. crassa as early as 1939 it was demonstrated that protoperithecia could be developed in the absence of the opposite mating type (Lindegren, 1939) but without the simultaneous development of the ascogenous hyphae. Hence, it appears that at least two genes are involved in the development of a fertile protoperithecium.

In order to make a thorough study of the sexual process in N. crassa, Vigfusson (1969) employed male sterility mutants. By means of ultraviolet irradiation of Neurospora crassa macroconidia and appropriate selection procedures, 32 mutant strains exhibiting male sterility

were isolated. Both mating types were irradiated and, based on the total number of isolates tested the *A* mating type showed a 5-fold greater frequency of mutation to male sterility than the *a* mating type. The mutants exhibited a wide spectrum of sexual behavior patterns when used as the male parent in crosses with a wild type strain indicating that the sexual development cycle is blocked at various stages in the different strains.

On the basis of complementation and intergenic recombination data and limited cytological observations, 4 genes were reported by Vigfusson which block 'early' stages of sexual development (i.e., prior to karyogamy). Similarly, at least 3 genes were indicated which showed blocks at later stages of development. In addition, a gene controlling female fertility was found in 5 out of 32 strains tested, the mutant gene resulting in complete female sterility. This gene segregated independently from male sterile genes which block early stages of the sexual cycle.

The aim of this investigation concerns the mechanics of genetic transmission of the sterility phenomena in *N.crassa*. Although Vigfusson (*loc. cit.*) found a Mendelian inheritance for male and female sterility in preliminary tests, no critical three- and four- point crosses were performed by him. The present thesis reports on these latter tests and on the basis of segregation data predicts the mapping of several sterility genes within the genome of *N.crassa*.

MATERIALS AND METHODS

1. Strains:

All strains of *Neurospora crassa* used in this study were supplied by the Fungal Genetics Stock Center, Dartmouth College, New Hampshire. Origin and description of stocks obtained for mapping are given in Table I. Stocks referred to in Table II are *leu-3* or *pan-1*, male sterility mutants (UV-induced) isolated by Dr. N.V. Vigfusson (1969). *Fl* strains were employed as tester strains for mating type and sterility determination.

2. Media:

(a) *Complete medium*. All cultures were maintained on standard complete medium consisting of Vogel's Medium N (Vogel; 1956) supplemented with 0.5% yeast extract, 0.5% casein hydrolysate, 0.1% standard vitamin solution and 0.005% tryptophan. Two per cent agar was used to solidify the medium.

TABLE I. DESCRIPTION OF STOCK CULTURES USED FOR MAPPING*

FGSC Stock No.	Marker	Allele No.	Linkage Group	Mating Type	Remarks
533	79a St. Lawrence Standard			*a*	Wild type.
103	*fr*	B110	IL	*A*	Visible marker.
1124	*leu*-3	R-156	IL	*A*	Leucine auxotroph
806	*crisp*	R2360	IL	*a*	Visible marker.
301	*met*-6	35809	IR	*a*	Methionine auxotr
267	*thi*-1	56501	IR	*a*	Thiamine auxotrop
45	*fl*	Lindegren (L)	IIR	*A*	(Fluffy, non-conid (fertile perithecia
46	*fl*	"	IIR	*a*	(parent.
239	*sc*, *tryp*-1	5801, 10575	IIIC, R	*a*	Tryptophan auxotr scumbo visible ma
71	*pan*-1	5531	IVR	*A*	(Pantothenic (acid
494	*pan*-1	5531	IVR	*a*	(auxotroph
94	*lys*-1	33933	VL	*a*	Lysine auxotroph.
411	*pab*-1	1633	VR	*a*	p-aminobenzoic ac auxotroph.
453	*ad*-8	Y-152-M7	VIL	*a*	Aderine auxotroph
134	*tryp*-2	45302	VIR	*a*	Tryptophan auxotr
	nic-3, *wc*		VIIL, C	*a*	Nicotinamide auxo and visible marke
	met-7	4894	VIIR	*a*	Metionine auxotro
281	*al*-2, *fl*, *sc*, *pan*-1, *inos*, *ylo*, *nt*	15300, L, 5801, 5531, 37401, Y30539$_y$, C86	all 7	*a*	Perkins (1959) multiple linkage tester stock.

*(Barratt and Ogata, 1962, 1968)

TABLE II. DESCRIPTION OF MALE STERILITY MUTANTS*

Isolate Number	Parental Strain	Mating Type	Description
7232	*leu*-3	*A*	Abundant small brown protoperithecia with occasional mature perithecia (0-10 per plate) which contain abundant normal spores.
8455	*leu*-3	*A*	
8553	*leu*-3	*A*	
10299	*leu*-3	*A*	
10710	*leu*-3, *fst*, *lg*	*A*	8553 produces a slightly greater number of mature perithecia (20-30) per plate
10710-1	*leu*-3	*A*	All mutations were UV-induced
10718	*leu*-3	*A*	
5538	*pan*-1	*A*	
16009	*pan*-1	*A*	

*) Vigfusson, 1969.

(b) Crossing medium. Standard crossing medium was used for all crosses (Westergaard and Mitchell 1947). Biochemical requirements were added as necessary in the amount of 50 mg/1000 ml. medium.

(c) Sorbose medium. This medium causes restricted colonial growth (Tatum *et al.*, 1949) and was used in tests for genetically determined biochemical requirements. The medium was prepared by substituting 90% of the sucrose in the medium by sorbose. Again, where necessary, biochemical requirements were added in the amount of 50 mg./ 1000 ml. medium.

3. Methods:

(a) Procedure for crossing. All crosses were carried out at 25°C using the male sterile mutants as the protoperithecial parent. To ensure maximum production of spores, fertilization was carried out in the following manner: Conidial (approximately 10^6 conidia/ml.) or mycelial (for non-conidiators) suspensions of sterile water (and 50 mg./ml. of the specific biochemical requirement of auxotrophic strains) were made from fresh cultures of the spermatial parent. One ml. aliquots of the suspension were added to week-old cultures of the perithecial strain. One week later another 1 ml. aliquot of a fresh suspension was added. A minimum of eight plates were made at any one time for a cross. Often many crosses had to be repeated two or three times due to lack of spore-shedding ascospores. Spores were allowed to mature for a minimum of three weeks before isolation.

(b) Isolation of ascospores. A platinum loop dipped in 1-2% calcium chloride solution was used to collect ascospores from the lid of a petri dish after which they were spread over a 6% agar surface in

such a manner that they were optimally spaced for future isolation. Ejection of ascospores from perithecia was poor in most crosses and often perithecia had to be dissected by squeezing with forceps in order to free the spore content. This latter system worked very well for the isolation of tetrads. Isolation was accomplished under 60X magnification using a sharp flat steel blade. Spores were lifted directly off the surface of the agar with the blade and transferred to a 50 ml. tube of complete media. Spores were induced to germinate by heat-shocking at 60^{o}C for 30 minutes and allowed to germinate at 30^{o}C.

(c) Linkage detection. All attempts to obtain fertile crosses between the male sterile mutants and the multiple linkage-tester stocks of Perkins (1959) failed. Multiple marker stocks containing two markers produced only ascospores in those cases in which the linkage tester strain carried at least one morphological marker. However, even then, segregation for parental markers was not always found to be random. Consequently each linkage group had to be tested separately, one marker at a time.

(d) Scoring of markers. Tests for male sterility and mating type were carried out by inoculating a small amount of conidia onto one of the four marked sectors of paired fl-a and fl-A protoperithecial plates (Smith, 1962). Consequently four different isolates can be accommodated per petri dish which in the experience of the present author seems to be the maximum number of isolates per plate in order to eliminate doubts in sterility and fertility scores. The plates were examined after 12-14 days incubation at 25^{o}C. Biochemical requirements of the isolates were scored on solidified sorbose media in petri dishes, divided into

quarters twenty-four hours after incubation at 30°C. The inoculations were marked either to be prototorphic or auxotrophic. Subsequently, sorbose agar medium (45°C) containing the necessary biochemical requirement was poured onto the center of the plate in such a manner that the media spread outwards covering the location of initial inoculation. After 24 hours of incubation the inocula were checked for resulting growth due to supplement of the biochemical requirement in the cover layer of sorbose agar medium. For slow-growing isolates (such as crisp) 36 hours of incubation was necessary in order to obtain satisfactory growth.

Results

The following male sterile mutants, all carrying either the biochemical requirement for leucine (*leu*-3) or for pantothenic acid (*pan*-1), were used in two-, three-, or four-point crosses to determine linkage relations of the male sterility genes with markers of known linkage groups:

8455 *A*, *leu*-3, *ster*
8553 *A*, *leu*-3, *ster*
10718 *A*, *leu*-3, *ster*
10710 *A*, *leu*-3, *ster*, *fr*, *lg*
10710-1 *A*, *leu*-3, *ster*
10299 *A*, *leu*-3, *ster*
7232 *A*, *leu*-3, *ster*
5538 *A*, *pan*-1, *ster*
16009 *A*, *pan*-1, *ster*

All crosses were characterized by the production of a large number of aborted spores, erratic germination percentages and repair of biochemical requirements in time. Only in a few cases(8455 *A*, *leu*-3, *ster* x 4894 *a*, *met*-7; 8455 *A*, *leu*-3, *ster* x 45302 *a*,*tryp*-2 and 8455 A, *leu*-3, *ster* x Y 3188, P 829 *a*, *nic*-3, *wc*) tetrad analysis could be carried out. Single strand analysis (random spore analysis) was used for establishing segregation ratios in all crosses.

(i) *Recombination data for mutant* 8455 *A*, *leu*-3, *ster*.

The male sterile strain 8455 *A*, *leu*-3, *ster* was used as protoperithecial parent in the following crosses:

Table III. RECOMBINATION DATA FOR MUTANT 8455

Cross: 8455 A, leu-3, ster x	Linkage Group	Germin. %	Recombination of sterility with marker				Recom. %	Recombination of sterility with mt (A vs. a)				Recom. %
			- ster -	- ster +	+ ster -	+ ster +		a ster -	a ster +	A ster -	A ster +	
fl	II R	55	22	33	20	19	44	21	31	21	21	44
pan-1	IV R	80	19	19	25	14	43	19	22	25	11	39
met-7	VII R	53	9	40	30	21	30	16	28	23	33	49
	VII R	72	16	34	37	13	29	23	22	30	25	48
nic-3	VII L	64	1	89	103	2	2	54	48	50	43	49
Pooled data for recombination between mt and ster								133	151	149	130	47

Table III (continued)

Tetrad analysis

Cross: 8455 A, leu-3, ster x	Linkage	Germin.	Recombination of sterility with marker. Ditypes			Distr.	Recom. %	Recombination of sterility with mt (A vs. a)			Distr.	Recom. %
			P	R	T			P	R	T		
tryp-2	VI C	57	0	2	5	N	64	1	2	4	N	57
met-7	VII R	61	18	0	6	L	12**	5	5	12	F	50
nic-3*	VII L	75	45	0	0	L	0**	11	12	22	F	50
wc*	VII R	75	29	0	16	L	18**	17	14	14	F	47
Pooled data for recombination between mt and ster								34	33	52		50

*) double marker parent
**) significant at 0.05 level

8455 *A*, *leu*-3, *ster* x L *a*, *fl*

8455 *A*, *leu*-3, *ster* x 5531 *a*, *pan*-1

8455 *A*, *leu*-3, *ster* x 4894 *a*, *met*-7

8455 *A*, *leu*-3, *ster* x Y 3188, P 829 *a*, *nic*-3, *wc*

8455 *A*, *leu*-3, *ster* x 45302 *a*, *tryp*-2

The results obtained are summarized in Table III. Linkage between *ster* and *met*-7 and between *ster* and *nic*-3 as well as between *ster* and *wc* was detected.

From crosses between 8455 *A*, *leu*-3, *ster* x Y 31881, P 829 *a*, *nic*-3, *wc* it became evident that *ster* is very closely linked to *nic*-3 (recombination frequency: 0%). *wc* (recombination frequency: 18%) and *met*-7 (recombination frequency: 13%) are located at some distance from *ster*. A complete absence of Tetratypes and Non-Parental Ditypes was noted for the segregation of *ster* and *nic*-3 in the cross 8455 *A*, *leu*-3, *ster* x Y 3188, P 829 *a*, *nic*-3, *wc*.

Single strand analysis of the remainder of crosses confirmed the tetrad analysis data with regard to linkage between *ster* and *met*-7 (recombination frequency: 29% and 30% respectively) and between *ster* and *nic* (recombination frequency:2%). Due to the close linkage between *ster* and *nic*-3 and the absence of suitable markers in the left arm of chromosome VII, no attempt was made to map *ster* relative to the location of the marker *nic*-3.

Tetrad analysis data as well as single strand analysis data were analysed for possible linkage between *ster* and *mt* (mating type: *A* *vs*. *a*). In all crosses examined no linkage between *ster* and *mt* was evident (pooled data tetrad analysis: 47% recombination; pooled data single strand analysis; 51% recombination).

Table IV. RECOMBINATION DATA FOR MUTANT 8553

Cross: 8553 A, leu-3, ster x	Linkage Group	Germin. %	Recombination of sterility with marker				Recom. %	Recombination of sterility with mt (A vs. a)				Recom. %
			- ster -	- ster +	+ ster -	+ ster +		a ster -	a ster +	A ster -	A ster +	
fr	I L	-	18	62	55	37	32	10	63	63	36	28***
leu-3*	I L	-	184	130	119	226	38	-	-	-	-	-
cr	I C	50	69	140	101	105	42	65	150	106	94	38***
met-6	I R	61	20	21	31	20	44	19	28	33	12	35***
thi-1	I R	65	17	41	69	31	30	30	35	56	37	42
fl	II R	24	9	13	6	8	47	7	12	8	9	45
sc**	III C	97	15	15	30	18	42	15	27	30	6	27***
tryp-1**	III R	97	21	16	24	17	49	-	-	-	-	-
pan-1	IV R	41	all pantothenic acid independent					5	22	16	7	24***
lys-1	V C	94	30	25	14	24	58	7	44	37	5	13***
pab-1	V R	99	11	35	22	32	43	21	38	12	29	50
ad-8	VI L	45	9	13	9	14	51	3	26	15	1	9***
tryp-2	VI C	95	11	20	28	36	50	11	30	28	26	39
met-7	VII R	93	30	15	24	24	57	16	27	38	12	30***
Pooled data for recombination between mt and ster								209	502	442	274	34***

*) pooled data of fr, St.Lawrence and cr crosses
**) double marker parent
***) significant at 0.05 level

(ii) Recombination data for mutant 8553 A, leu-3, ster.

The male sterile, leucine requiring mutant strain 8553 A, leu-3, ster was crossed to marker strains representing all the linkage groups of N. crassa. The following crosses were successful in producing sufficient ascospores for single strand analysis:

8553 A, leu-3, ster x B 110 a, fr

8553 A, leu-3, ster x St.Lawrence a

8553 A, leu-3, ster x R 2360 a, cr

8553 A, leu-3, ster x 35809 a, met-6

8553 A, leu-3, ster x 56501 a, thi-1

8553 A, leu-3, ster x L a, fl

8553 A, leu-3, ster x 5801, 10575 a, sc, tryp-1

8553 A, leu-3, ster x 5531 a, pan-1

8553 A, leu-3, ster x 33933 a, lys-1

8553 A, leu-3, ster x 1633 a, pab-1

8553 A, leu-3, ster x Y-153-M7 a, ad-8

8553 A, leu-3, ster x 45302 a, tryp-2

8553 A, leu-3, ster x 4894 a, met-7

Single strand analysis of cross 8553 A, leu-3 x B 110 a, fr indicated possible linkage between ster and fr (recombination frequency: 32%) and ster and thi-1 (recombination frequency: 30%). Both markers, fr as well as thi-1, are located on chromosome I although on different arms (Table IV).

The analysis of data with regard to possible linkage between ster and mt (mating type: A vs. a) showed various strengths of linkage in the following crosses (Table IV):

8553 A, leu-3, ster x B 110 a, fr (recombination frequency: 28%)

8553 *A*, *leu*-3, *ster* x R 2360 *a*, *cr* (recombination frequency: 28%)

8553 *A*, *leu*-3, *ster* x 35809 *a*, *met*-6 (recombination frequency: 38%)

8553 *A*, *leu*-3, *ster* x 5801, 10575 *a*, *sc*, *tryp*-1 (recombination frequency: 27%)

8553 *A*, *leu*-3, *ster* x 5531 *a*, *pan*-1 (recombination frequency: 24%)

8553 *A*, *leu*-3, *ster* x 33933 *a*, *lys*-1 (recombination frequency: 13%)

8553 *A*, *leu*-3, *ster* x Y-153-M7 *a*, *ad*-8 (recombination frequency: 9%)

8553 *A*, *leu*-3, *ster* x 4894 *a*, *met*-7 (recombination frequency: 30%)

In pooling the results of all crosses a recombination frequency of 34% was obtained for the markers *ster* and *mt*.

The cross 8553 *A*, *leu*-3, *ster* x 5531 *a*, *pan*-1 did not yield pantothenic acid requiring progeny as expected. On repeating this cross similar results were obtained.

(iii) *Recombination data for mutant* 10178 *A*, *leu*-3, *ster*.

The male sterile, leucine requiring strain 10178 *A*, *leu*-3, *ster* was crossed to several markers representing the seven linkage groups of *N. crassa*:

10718 *A*, *leu*-3, *ster* x L *a*, *fl*

10718 *A*, *leu*-3, *ster* x 5801, 10575 *a*, *sc*, *tryp*-1

10718 *A*, *leu*-3, *ster* x 5531 *a*, *pan*-1

10718 *A*, *leu*-3, *ster* x 1633 *a*, *pab*-1

10718 *A*, *leu*-3, *ster* x 45302 *a*, *tryp*-2

10718 *A*, *leu*-3, *ster* x Y-153-M7 *a*, *ad*-8

10718 *A*, *leu*-3, *ster* x 4894 *a*, *met*-7

In all crosses recombination frequencies were determined by means of single strand analysis. The recombination frequency between *ster* and the *leu*-3 marker(allele Nr. R 156) was calculated from the

Table V. RECOMBINATION DATA FOR MUTANT 10718

Cross: 10718 A, leu-3 ster x	Linkage Group	Germin. %	Recombination of sterility with marker				Recom. %	Recombination of sterility with mt (A vs. a)				Recom. %
			- ster -	- ster +	+ ster -	+ ster +		a ster -	a ster +	A ster -	A ster +	
leu-3*	I L	-	92	46	56	99	35**	-	-	-	-	
fl	II R	35	12	8	5	10	63	6	9	11	9	43
sc***	III C	78	26	11	15	18	63	21	21	20	3	41
tryp-1***	III R	78	28	15	13	14	60	-	-	-	-	-
pan-1	IV R	78	20	25	31	23	45	22	30	29	18	40
pab-1	V R	-	32	31	26	30	53	25	37	33	24	41
tryp-2	VI C	93	14	24	24	31	48	12	33	26	22	37**
ad-8	VI L	80	22	11	21	26	60	12	28	31	9	26**
met-7	VII R	58	19	22	26	20	45	12	42	29	4	18**
Pooled data for recombination between mt and ster								110	200	179	94	35**

*) leu-3 requirement tested in isolates from met-7, tryp-2 and pab-1 crosses
**) significant at 0.05 level
***) double marker parent

Table VI. RECOMBINATION DATA FOR MUTANT 10710

Cross:

10710 *A*, *leu*-3, *ster*, *fst*, *lg* x St.Lawrence *a*

		Number	Per cent
parentals:	*ster*, *fst*, *lg*	48	78
	ster^{+}, *fst*$^{+}$, *lg*$^{+}$	49	
single crossovers:	*ster*, *fst*$^{+}$, *lg*$^{+}$	11	12
	X		
	ster^{+}, *fst*, *lg*	3	
	ster, *fst*, *lg*$^{+}$	6	5
	X		
	ster^{+}, *fst*$^{+}$, *lg*	1	
double crossovers:	*ster*, *fst*$^{+}$, *lg*	2	5
	X X		
	ster^{+}, fst, lg^{+}	4	

Distances:

	ster - *fst*	16.2 m.u.
	fst - *lg*	10.5 m.u.
	ster - *lg*	26.7 m.u.

Interference: -6.8

Map: ster fst lg

16.2 10.5

26.7

recombination data of the following crosses:(Table V):

10718 *A*, *leu*-3, *ster* x 1633 *a*, *pab*-1
10718 *A*, *leu*-3, *ster* x 45302 *a*, *tryp*-2
10718 *A*, *leu*-3, *ster* x 4894 *a*, *met*-7

The segregation data obtained from the above crosses indicate linkage between *ster* and *leu*-3 of linkage group I (recombination frequency: 35%).

Linkage between *ster* and *mt* (*A* *vs*. *a*) was found to exist in the following crosses:

10718 *A*, *leu*-3, *ster* x 45302 *a*, *tryp*-2 (recombination frequency: 37%)
10718 *A*, *leu*-3, *ster* x Y-153-M7 *a*, *ad*-8 (recombination frequency: 26%)
10718 *A*, *leu*-3, *ster* x 4894 *a*, *met*-7 (recombination frequency: 19%)

The recombination frequency for *ster* and *mt* , based on pooled data of all the crosses, amounted to 35% (Table V).

(iv) *Recombination data for mutant* 10710 *A*, *leu*-3, *ster*, *fst*, *lg*.

The original mutant 10710 is characterized by partial male sterility(*ster*) and complete female sterility(*fst*). In addition, the strain carries a requirement for leucine(*leu*-3) as well as a mutation which causes 'limited growth'(*lg*), i.e., the number of macroconidia produced on a slant of solidified medium was found to be drastically reduced when compared to the standard wild type St.Lawrence strain. Its phenotype resembles that of the mutant *fluffy*(*fl*) except for the fact that normal orange-red pigment is present in the *lg* strain.

The strain 10710 *A*, *leu*-3, *ster*, *fst*, *lg* was crossed to St. Lawrence wild type strain mating type *a*. Single strand analysis of the progeny of this cross (Table VI) indicated that *ster* and *fst* recombine with a frequency of 16% which indicates linkage between these two markers.

Table VII. RECOMBINATION DATA FOR MUTANT 10710-1

Cross: 10710-1 A, leu-3, ster x	Linkage Group	Germin. %	Recombination of sterility with marker				Recom. %	Recombination of sterility with mt (A vs. a)				Recom. %
			- ster -	- ster +	+ ster -	+ ster +		a ster -	a ster +	A ster -	A ster +	
fl	II R	62	40	33	27	24	51	31	32	36	25	45
sc*	III C	84	14	14	32	7	31**	16	15	30	6	33**
tryp-1	III R	84	15	12	31	9	36**	-	-	-	-	-
pan-1	IV R	39	33	21	25	18	47	28	33	25	28	49
lys-1	V C	91	34	14	25	18	57	23	24	36	8	34**
pab-1	V R	91	25	19	24	22	52	23	24	26	17	44
ad-8	VI L	87	16	24	24	23	45	13	31	27	16	33**
tryp-2	VI C	92	20	22	22	28	52	14	33	28	17	34**
met-7	VII R	52	22	19	16	26	58	11	31	27	14	30**
Pooled data for recombination between mt and ster								159	223	235	131	38.8**

*) double marker parent

**) significant at the 0.05 level

Female sterility (*fst*) and *lg* were found to recombine with a frequency of 11%, whereas *ster* is linked to *lg* (recombination frequency: 17%, expected recombination frequency: 27%). All three genes (*ster*, *fst*, *lg*) are located in the same linkage group, wherever this may be. No linkage was detected between these genes (*ster*, *fst*, *lg*) and *mt* (*A* *vs*. *a*) (Table VI).

(v) *Recombination* *data* *for* *mutant* 10710-1 *A*, *leu*-3, *ster*.

Mutant strain 10710-1 *A*, *leu*-3, *ster* is a male sterile, leucine requiring strain and is a single strand isolate of the cross:

10710-1 *A*, *leu*-3, *ster*, *fst*, *lg* x St.Lawrence *a*.

Strain 10710-1 *A*, *leu*-3, *ster* was mated to the following marker strains:

10710-1 *A*, *leu*-3, *ster* x L *a*, *fl*

10710-1 *A*, *leu*-3, *ster* x 5801, 10575 *a*, *sc*, *tryp*-1

10710-1 *A*, *leu*-3, *ster* x 5531 *a*, *pan*-1

10710-1 *A*, *leu*-3, *ster* x 33933 *a*, *lys*-1

10710-1 *A*, *leu*-3, *ster* x 1633 *a*, *pab*-1

10710-1 *A*, *leu*-3, *ster* x Y-153-M7 *a*, *ad*-8

10710-1 *A*, *leu*-3, *ster* x 45302 *a*, *tryp*-2

10710-1 *A*, *leu*-3, *ster* x 4894 *a*, *met*-7

Single strand analysis of the above crosses (Table VII) revealed linkage between *ster* and *sc* (recombination frequency: 31%) and between *ster* and *tryp*-1 (recombination frequency: 36%).

Linkage between *ster* and *mt* (*A* *vs*.*a*)occurred in the following crosses:

10710-1 *A*, *leu*-3, *ster* x 5801, 10575 *a*, *sc*, *tryp*-1(recombination frequency:33%)

10710-1 *A*, *leu*-3, *ster* x 33933 *a*, *lys*-1(recombination frequency:34%)

10710-1 *A*, *leu*-3, *ster* x Y-153-M7 *a*, *ad*-8(recombination frequency:33%)

Table VIII. RECOMBINATION DATA FOR MUTANT 10299

Cross: 10299 A, leu-3, ster x	Linkage Group	Germin. %	Recombination of sterility with marker				Recom. %	Recombination of sterility with mt (A vs. a)				Recom. %
			- ster -	- ster +	+ ster -	+ ster +		a ster -	a ster +	A ster -	A ster +	
leu-3	I L	83	50	39	8	19	41	13	47	45	11	21**
fl	II R	12	6	1	6	5	60	4	5	8	1	28**
sc*	III C	91	28	8	25	12	55	27	15	26	5	44
tryp-1*	III R	91	18	9	24	11	40	-	-	-	-	-
pan-1	IV R	0	-	-	-	-	-	-	-	-	-	-
pab-1	V R	77	14	24	29	21	40	16	30	27	15	35**
ad-8	VI L	100	28	27	24	21	49	16	28	36	20	36**
tryp-2	VI C	95	20	29	17	34	54	11	34	38	17	28**
met-7	VII R	95	24	21	30	20	46	15	32	39	9	25**
Pooled data for recombination between mt and ster								102	191	219	78	30.5**

*) double marker parent

**) significant at the 0.05 level

10710-1 A, leu-3, ster x 45302 a, tryp-2(recombination frequency:34%)

10710-1 A, leu-3, ster x 4894 a, met-7(recombination frequency:30%)

Taking into account all crosses (pooled data) a recombination frequency of 39% for ster and mt can be calculated.

(vi) Recombination data for mutant 10299 A, leu-3, ster.

The following crosses between the male sterile, leucine requiring strain 10299 A, leu-3, ster and marker strains for all linkage groups were analysed:

10299 A, leu-3, ster x L a, fl

10299 A, leu-3, ster x 5801, 10575 a, sc, tryp-1

10299 A, leu-3, ster x 5531 a, pan-1

10299 A, leu-3, ster x 1633 a, pab-1

10299 A, leu-3, ster x Y-153-M7 a, ad-8

10299 A, leu-3, ster x 45302 a, tryp-2

10299 A, leu-3, ster x 4894 a, met-7

Single strand analysis of the progeny obtained (Table VIII) from the above mentioned crosses, did not reveal linkage between ster and the different biochemical and morphological markers. Analysis for possible linkage between ster and mt (A vs.a) yielded a positive result for the following crosses:

10299 A, leu-3, ster x St.Lawrence a(recombination frequency:21%)

10299 A, leu-3, ster x L a, fl(recombination frequency:28%)

10299 A, leu-3, ster x 1633 a, pab-1(recombination frequency:35%)

10299 A, leu-3, ster x Y-153-M7 a, ad-8(recombination frequency:36%)

10299 A, leu-3, ster x 45302 a, tryp-2(recombination frequency:28%)

10299 A, leu-3, ster x 4894 a, met-7(recombination frequency:25%)

The average recombination frequency for all crosses (pooled

Table IX. RECOMBINATION DATA FOR MUTANT 7232

Cross: 7232 A,leu-3, ster x	Linkage Group	Germin. %	Recombination of sterility with marker				Recom. %	Recombination of sterility with mt (A vs. a)				Recom. %
			- ster -	- ster +	+ ster -	+ ster +		a ster -	a ster +	A ster -	A ster +	
cr	I C	88	23	23	26	17	45	26	21	23	19	50
fl	II R	89	34	20	24	12	51	23	20	35	12	39**
sc*	III C	88	13	10	28	19	46	13	23	28	6	27**
tryp-1*	III R	88	15	13	26	16	44	-	-	-	-	-
pan-1	IV R	43	all offspring pantothenic acid independent									
lys-1	V C	92	19	20	26	22	47	13	33	32	9	25**
pab-1	V R	99	20	20	29	30	50	17	28	32	22	39**
ad-8	VI L	88	24	22	23	19	49	17	30	30	11	32**
tryp-2	VI C	98	20	29	20	29	50	15	42	25	16	32**
met-7	VII R	99	23	22	21	33	56	14	34	30	21	35**
Pooled data for recombination between mt and ster								138	231	235	116	35.3**

*) double marker parent

**) significant at the 0.05 level

data) for *ster* and *mt* amounted to 31%.

(vii) *Recombination data for mutant* 7232 *A*, *leu*-3, *ster*.

Strain 7232 *A*, *leu*-3, *ster* is a male sterile, leucine requiring mutant. This mutant was employed in the following crosses with marker strains representing all the different linkage groups of *N. crassa*, in order to determine possible linkage relationships:

7232 *A*, *leu*-3, *ster* x R2360 *a*, *cr*
7232 *A*, *leu*-3, *ster* x L *a*, *fl*
7232 *A*, *leu*-3, *ster* x 5801, 10575 *a*, *sc*, *tryp*-1
7232 *A*, *leu*-3, *ster* x 5531 *a*, *pan*-1
7232 *A*, *leu*-3, *ster* x 33933 *a*, *lys*-1
7232 *A*, *leu*-3, *ster* x 1633 *a*, *pab*-1
7232 *A*, *leu*-3, *ster* x Y-153-M7 *a*, *ad*-8
7232 *A*, *leu*-3, *ster* x 45302 *a*, *tryp*-2
7232 *A*, *leu*-3, *ster* x 4894 *a*, *met*-7

Single strand analysis of the offspring(Table IX) of the above crosses did not indicate any linkage relationship between *ster* and any of the known markers.

All the viable progeny of the cross 7232 *A*, *leu*-3, *ster* x 5531 *a*, *pan*-1 were found to be prototrophs: no Mendelian segregation for *pan*-1 was observed.

Single strand analysis of the following crosses revealed linkage between *ster* and *mt*(*A vs.a*):

7232 *A*, *leu*-3, *ster* x L *a*, *fl*(recombination frequency:39%)
7232 *A*, *leu*-3, *ster* x 5801, 10575 *a*, *sc*, *tryp*-1(recombination frequency:27%)
7232 *A*, *leu*-3, *ster* x 33933 *a*, *lys*-1(recombination frequency:25%)
7232 *A*, *leu*-3, *ster* x 1633 *a*, *pab*-1(recombination frequency:39%)

7232 *A*, *leu*-3, *ster* x Y-153-M7 *a*, *ad*-8(recombination frequency:32%)

7232 *A*, *leu*-3, *ster* x 45302 *a*, *tryp*-2(recombination frequency:32%)

7232 *A*, *leu*-3, *ster* x 4894 *a*, *met*-7(recombination frequency:35%)

For pooled data the recombination frequency between *ster* and *mt* amounted to 35%.

(viii) *Recombination data for mutant* 5538 *A*, *pan*-1, *ster*.

Strain 5538 *A*, *pan*-1, *ster* is a male sterile, pantothenic acid requiring isolate. Single strand analysis was carried out on the progenies of the *ster* mutant and marker strains for all linkage groups of *N. crassa*(except for linkage groups III; the cross 5538 *A*, *pan*-1, *ster* x 5801, 10575 *a*, *sc*, *tryp*-1 did not yields ascospores):

5538 *A*, *pan*-1, *ster* x R2360 *a*, *cr*

5538 *A*, *pan*-1, *ster* x L *a*, *fl*

5538 *A*, *pan*-1, *ster* x 1633 *a*, *pab*-1

5538 *A*, *pan*-1, *ster* x Y-153-M7 *a*, *ad*-8

5538 *A*, *pan*-1, *ster* x 45302 *a*, *tryp*-2

5538 *A*, *pan*-1, *ster* x 4894 *a*, *met*-7

Single strand analysis of the progenies of the above crosses (Table X) indicated marginal linkage between *ster* and *cr* (40%), *ster* and *ad*-8 (39%) and, *ster* and *met*-7(38%). Analysis of the progenies of all crosses for possible linkage between *ster* and *mt*(*A* *vs*. *a*) indicated linkage in the following cases:

5538 *A*, *pan*-1, *ster* x R2360 *a*, *cr*(recombination frequency:36%)

5538 *A*, *pan*-1, *ster* x Y-153-M7 *a*, *ad*-8(recombination frequency:35%)

5538 *A*, *pan*-1, *ster* x 45302 *a*, *tryp*-2(recombination frequency:39%)

5538 *A*, *pan*-1, *ster* x 4894 *a*, *met*-7(recombination frequency:21%)

The average (pooled data) recombination frequency between *ster*

Table X. RECOMBINATION DATA FOR MUTANT 5538

Cross: 5538 A,pan-1, ster x	Linkage Group	Germin. %	Recombination of sterility with marker				Recom. %	Recombination of sterility with mt (A vs. a)				Recom. %
			- ster -	- ster +	+ ster -	+ ster +		a ster -	a ster +	A ster -	A ster +	
cr	I C	40	15	30	16	16	40	12	30	19	16	36*
fl	II R	37	24	24	15	14	49	15	16	24	22	48
pan-1**	IV R	all offspring pantothenic acid independent										
pab-1	V R	48	23	16	23	26	56	18	25	28	17	40
ad-8	VI L	41	12	25	22	18	39*	13	29	21	14	35*
tryp-2	VI C	42	21	16	16	22	58	14	23	23	15	39*
met-7	VII R	41	11	16	29	16	38*	7	24	33	8	21*
Pooled data for recombination between mt and ster												

*) significant at the 0.05 level
**) data from cr and fl crosses

Table XI. RECOMBINATION DATA FOR MUTANT 16009

Cross: 16009 A,pan-1, ster x	Linkage Group	Germin. %	Recombination of sterility with marker				Recom. %	Recombination of sterility with mt (A vs. a)				Recom. %
			- ster -	- ster +	+ ster -	+ ster +		a ster -	a ster +	A ster -	A ster +	
fl	II R	33	10	13	9	16	54	6	14	13	15	44
sc*	III C	40	19	3	10	8	68*	11	10	18	1	30**
tryp-1*	III R	40	18	3	11	8	65*	-	-	-	-	-
pan-1	IV R	Offspring from pab-1 and met-7 crosses all pantothenic acid independent										
pab-1	V R	43	17	6	27	19	52	22	14	22	11	48
ad-8	VI L	51	18	19	22	19	47	16	31	24	7	30**
tryp-2	VI C	0	-	-	-	-	-	-	-	-	-	-
met-7	VII R	51	14	20	21	25	48	10	33	25	12	28*
Pooled data for recombination between mt and ster								65	102	102	46	35.2*

*) double marker parent
**) significant at the 0.05 level

and *mt* amounted to 37%.

The crosses 5538 *A*, *pan*-1, *ster* x R2360 *a*, *cr* and
5538 *A*, *pan*-1, *ster* x L *a*, *fl* were analysed
for *pan*-1 segregants. Single strand analysis indicated that in both crosses the expected *pan*-1 genotypes were lacking (Table X).

(ix) *Recombination data for mutant* 16009 *A*, *pan*-1, *ster*.

Strain 16009 *A*, *pan*-1, *ster* is a male sterile, pantothenic acid requiring mutant and was used in the following crosses with marker strains of all linkage groups:

16009 *A*, *pan*-1, *ster* x L *a*, *fl*
16009 *A*, *pan*-1, *ster* x 5801, 10575 *a*, *sc*, *tryp*-1
16009 *A*, *pan*-1, *ster* x 1633 *a*, *pab*-1
16009 *A*, *pan*-1, *ster* x Y-153-M7 *a*, *ad*-8
16009 *A*, *pan*-1, *ster* x 45302 *a*, *tryp*-2
16009 *A*, *pan*-1, *ster* x 4894 *a*, *met*-7

Single strand analysis of the above cross (Table XI) yielded recombination values for *ster* and the known markers which were not indicative for linkage. Analysis of the cross 16009 *A*, *pan*-1, *ster* a 5801, 10575 *a*, *sc*, *tryp*-1 yielded a recombination value of 68% for the markers *ster* and *sc* and 65% for the markers *ster* and *tryp*-1.

The crosses 16009 *A*, *pan*-1, *ster* x 1633 *a*, *pab*-1 and,
16009 *A*, *pan*-1, *ster* x Y-153-157 *a*, *ad*-8
yielded no pantothenic requiring progeny.

Linkage between *ster* and *mt*(*A* *vs*.*a*) was evident from single strand analysis of the following crosses:

16009 *A*, *pan*-1, *ster* x 5801, 10575 *a*, *sc*, *tryp*-1(recombination frequency:30%)
16009 *A*, *pan*-1, *ster* x Y-153-M7 *a*, *ad*-8(recombination frequency:30%)

16009 A, pan-1, ster x 4894 a, met-7(recombination frequency:28%)

The average (pooled data) recombination frequency between ster and mt to 35%.

Discussion

Sterility in the Ascomycete Neurospora crassa is a common phenomenon and occurs in a spectrum of expressions. The original 'Dodge' strains may have exhibited a large degree of sterility. Lindegren (1936) as well as Moreau and Moruzi (1936) have reported on the presence of sterility in the early strains of the fungus. Genetics is indebted to Dr. Patricia St.Lawrence for the development of strains which in their wild type phenotype exhibit a high dgree of fertility. However, even these highly'purified'strains with their uniform genetic background often display sterility in connection with induced biochemical requirements. It is for this reason that intragenic recombination studies are often difficult to perform (Weijer, 1954). The pertinent question with regard to sterility relates to whether or not sterility phenomena in N. crassa are of a genetic nature. Vigfusson (1969) pointed out that on the basis of segregation and complementation data for sterility mutants of Neurospora in crosses with wild type progenitors, a genetic determination for sterility was evident for many of his mutants. In those cases of dubious genetic determination, evidence for cytoplasmic inheritance remained negative (see Appendix).

The present study confirms Vigfusson's observation of a genetic basis of sterility for all the mutants studies. For instance, the mutation male sterility (ster) of strain 8455 A, leu-3, ster is located in Linkage Group VII on the basis of the recombination frequency ster - nic-3 (Table III) a marker of Linkage Group VII. Since all male sterility mutants are phenotypically alike, it is tempting to assume that therefore male sterility in all mutants tested, concerns a genetic character and is inherited in a Mendelian fashion by subsequent progeny. Conclusive evidence for

genetic transmission of male sterility was obtained from the segregation data of the cross 10710 *A*, *leu*-3, *ster*, *fst*, *lg* x St.Lawrence *a* (Table V) in which linkage between *ster* and *fst* (female sterility) together with linkage between *ster* and *lg* (limited growth) was apparent. The very fact that in all crosses the male sterility factor segregates with male fertility(*ster*$^+$) in an one to one ratio lends support to the above assumption of a genetic basis for male sterility of all the mutants tested.

Except for mutant 8455 *A*, *leu*-3, *ster* all male sterility mutants showed linkage with the mating type (*mt*) locus, however the exact location of the sterility factor could not be deduced. The reason for this inconclusiveness with regard to a possible location of *ster* in linkage group I is understandable since not in all individual crosses the linkage relationship between *ster* and *mt* was evident (Table V).

In this respect, the results obtained with mutant 8553 *A*, *leu*-3, *ster* is of interest. In cross 8553 *A*, *leu*-3, *ster* x St.Lawrence *a* (treated as a two-point cross, thereby excluding double and triple crossovers,Table IV) a recombination frequency of 17% was obtained for *leu*-3 and *mt*, 27% for *mt* and *ster* and 37% for *leu*-3 and *ster*. A gene order

leu-3----------*mt*----------*ster*

is indicative.

In cross 8553 *A*, *leu*-3, *ster* x 35809 *a*, *met*-6 (treated as a two-point cross, Table IV) the following recombination frequencies were evident:

met-6 - *mt*.........26%

mt - *ster*..........35%

ster - *met*-6.......44%

indicating a gene order of

ster----------*mt*----------*met*-6

In cross 8553 *A*, *leu*-3, *ster* x R2360 *a*, *cr* a recombination frequency of 18% was obtained for *cr* and *mt*. *mt* and *ster* yielded a recombination frequency of 33%, whereas *ster* and *cr* recombined with a frequency of 39%. The gene order

ster----------*mt*----------*cr*

satisfy the data of this two-point cross.

In cross 8553 *A*, *leu*-3, *ster* x 56501 *a*, *thi*-1 (treated as a two-point cross, Table V) the following recombination frequencies were obtained:

thi-1 - *mt*..........50%

mt - *ster*..........42%

ster - *thi*-1........30%

Suggested gene order:

mt----------*ster*----------*thi*-1

In the case of the cross 8553 *A*, *leu*-3, *ster* x B 110 *a*, *fr*, *mt* and *fr* recombined with a frequency of 35%, *mt* and *ster* with a frequency of 27% and *fr* and *ster* with 32% (Table V).

A rather unsatisfactory gene order of

fr----------*ster*----------*mt*

can be deduced.

Hence, markers to the left of *mt* tend to place *ster* to the right of *mt* (in the case of *leu*-3 and perhaps in the case of *fr*), whereas markers to the right of *mt* (*met*-6 and *cr*) place *ster* to the left of *mt*. The outside marker *thi*-1 (and perhaps *fr*) do not conform to this rule.

Although there is no consistency in the map location of *ster* with reference to *mt*, there seems to be consistency of results for one and the same outside marker in different crosses. For instance the outside marker *leu*-3 in all the five crosses involving 8553 *A*, *leu*-3, *ster* places *ster* to the right of *mt*. The pooled data for the outside marker *leu*-3

Table XII. ANALYSIS OF THE RECOMBINATION DATA FROM THE CROSS:
8553 A, leu-3, ster x B 110 a, fr

Cross:

8553 A, leu-3, ster x B 110 a, fr

								Number	Per cent
parentals	fr$^+$,		leu-3,		ster,		A	48	52
	fr ,		leu-3$^+$,		ster$^+$,		a	42	
single crossovers	fr,		leu-3,		ster,		A	3	11
		X							
	fr$^+$,		leu-3$^+$,		ster$^+$,		a	15	
	fr$^+$,		leu-3,		ster$^+$,		a	6	10
				X					
	fr,		leu-3$^+$,		ster,		A	11	
	fr$^+$,		leu-3,		ster,		a	3	9
						X			
	fr,		leu-3$^+$,		ster$^+$,		A	13	
double crossovers	fr$^+$,		leu-3$^+$,		ster,		A	1	1
		X		X					
	fr,		leu-3 ,		ster$^+$,		a	0	
	fr$^+$,		leu-3,		ster$^+$,		A	14	10
				X		X			
	fr,		leu-3$^+$,		ster ,		a	4	
	fr$^+$,		leu-3$^+$,		ster$^+$,		A	2	1
		X				X			
	fr,		leu-3,		ster,		a	0	
triple crossovers	fr$^+$,		leu-3$^+$,		ster,		a	3	6
		X		X		X			
	fr,		leu-3,		ster$^+$,		A	7	

Table XII (continued)

Distances:		
	fr to *leu-3*	18 m.u.
	leu-3 to *ster*	27 m.u.
	ster to *mt*	27 m.u.
	mt to *leu-3*	54 m.u.
	mt to *fr*	71 m.u.
	fr to *ster*	55 m.u.

Map:

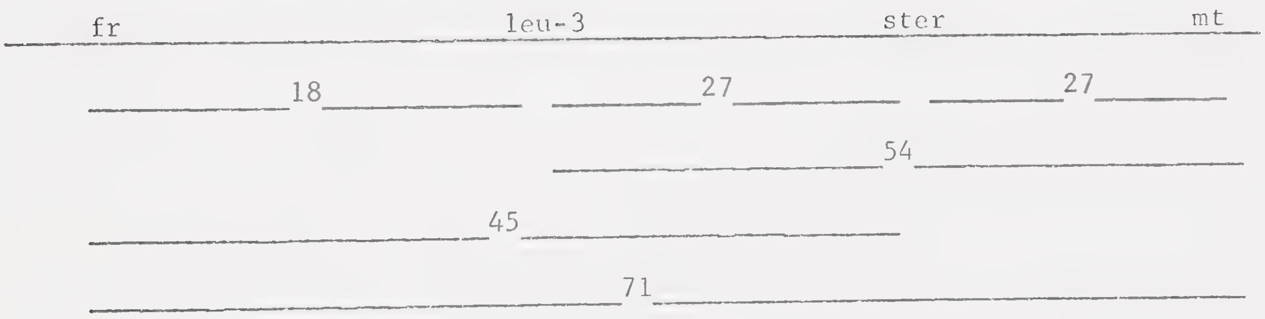


Table XIII. ANALYSIS OF THE RECOMBINATION DATA FROM THE CROSS:
8553 A, leu-3, ster x R2360 a, cr

Cross:

8553 A,leu-3,ster x R 2360 a,cr

								Number	Per cent
parentals	leu-3,		ster,		A,		cr$^+$	57	41
	leu-3$^+$,		ster$^+$,		a,		cr	77	
single crossovers	leu-3,		ster,		A,		cr	10	9
						X			
	leu-3$^+$,		ster$^+$,		a,		cr$^+$	19	
	leu-3,		ster$^+$,		a,		cr	10	7
		X							
	leu-3$^+$,		ster,		A,		cr$^+$	13	
	leu-3,		ster,		a,		cr	8	6
				X					
	leu-3$^+$,		ster$^+$,		A,		cr$^+$	12	
double crossovers	leu-3$^+$,		ster,		A,		cr	1	0 (0.3)
		X				X			
	leu-3 ,		ster$^+$,		a,		cr$^+$	0	
	leu-3$^+$,		ster,		a,		cr	42	29
		X		X					
	leu-3 ,		ster$^+$,		A,		cr$^+$	52	
	leu-3 ,		ster ,		a,		cr$^+$	1 .	0 (0.3)
				X		X			
	leu-3$^+$,		ster$^+$,		A,		cr	0	
triple crossovers	leu-3 ,		ster$^+$,		A,		cr	14	8
		X		X		X			
	leu-3$^+$,		ster ,		a,		cr$^+$	11	

Table XIII(continued)

Distances:		
	cr to *mt*	17 m.u.
	mt to *ster*	43 m.u.
	ster to *leu-3*	44 m.u.
	mt to *leu-3*	87 m.u.
	ster to *cr*	60 m.u.
	leu-3 to *cr*	104 m.u.

Map:

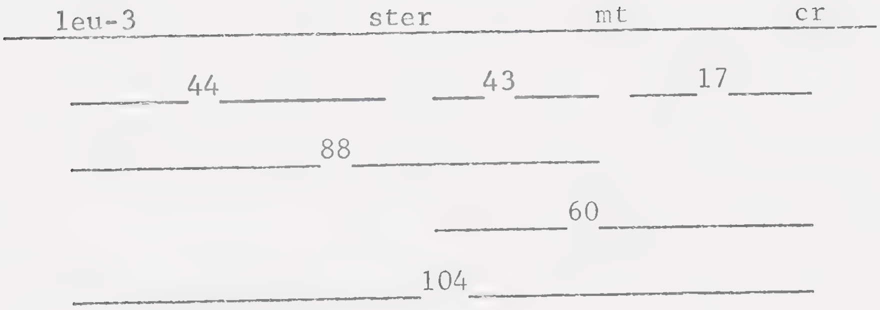


Table XIV. ANALYSIS OF THE RECOMBINATION DATA FROM THE CROSS:

8553 *A*, *leu*-3, *ster* x 56501 *a*, *thi*-1

Cross:

8553 *A*,*leu*-3,*ster* x 56501 *a*,*thi*-1

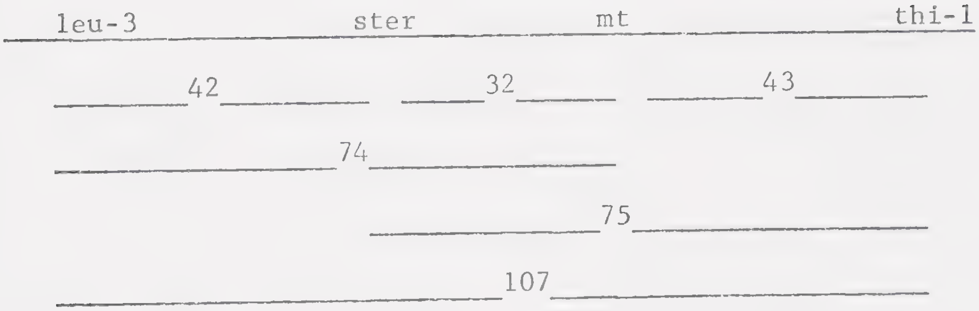
		Number	Per cent
parentals	*leu*-3, *ster*, *A*, *thi*$^+$ *leu*-3$^+$, *ster*$^+$, *a*, *thi*	41	41
single crossovers	*leu*-3, *ster*, *A*, *thi* X *leu*-3$^+$, *ster*$^+$, *a*, *thi*$^+$	8	8
	leu-3, *ster*$^+$, *a*, *thi* X *leu*-3$^+$, *ster*, *A*, *thi*$^+$	6	6
	leu-3, *ster*, *a*, *thi* X *leu*-3$^+$, *ster*$^+$, *A*, *thi*$^+$	1	1
double crossovers	*leu*-3$^+$, *ster*, *A*, *thi* X X *leu*-3, *ster*$^+$, *a*, *thi*$^+$	13	13
	leu-3$^+$, *ster*, *a*, *thi* X X *leu*-3 , *ster*$^+$, *A*, *thi*$^+$	9	9
	leu-3, *ster*, *a*, *thi*$^+$ X X *leu*-3$^+$, *ster*$^+$, *A*, *thi*	8	8
triple crossovers	*leu*-3, *ster*$^+$, *A*, *thi* X X X *leu*-3$^+$, *ster*, *a*, *thi*$^+$	14	14

Table XIV(continued)

Distances:

thi-1 to mt	43 m.u.
mt to ster	32 m.u.
ster to leu-3	42 m.u.
mt to leu-3	74 m.u.
ster to thi-1	75 m.u.
leu-3 to thi-1	107 m.u.

Map:



yielded the following recombination frequencies:

leu-3 - *ster*..........35%

leu-3 - *mt*............17%

mt - *ster*.............33%

These frequencies are indicative for the gene order:

leu-3----------*mt*----------*ster*

The obvious conclusion to draw from the above results is to assume that the gene for male sterility(*ster*) is located between the markers *leu*-3 and *mt*. On the basis of this assumption the following four-point crosses:

8553 *A*, *leu*-3, *ster* x B 110 *a*, *fr* (Table XII)

8553 *A*, *leu*-3, *ster* x R2360 *a*, *cr* (Table XIII)

8553 *A*, *leu*-3, *ster* x 56501 *a*, *thi*-1 (Table XIV)

yielded the following recombination frequencies:

Outside marker *fr*	Outside marker *cr*	Outside marker *thi*-1
leu-3 - *ster*........27%	*leu*-3 - *ster*........44%	*leu*-3 - *ster*........42%
mt - *ster*...........27%	*mt* - *ster*...........43%	*mt* - *ster*...........32%
leu-3 - *mt*..........54%	*leu*-3 - *mt*..........87%	*leu*-3 - *ster*........74%
fr - *leu*-3..........18%	*cr* - *leu*-3.........104%	*thi*-1 - *leu*-3......107%
fr - *mt*.............71%	*cr* - *mt*.............17%	*thi*-1 - *mt*..........43%
fr - *ster*...........45%	*cr* - *ster*...........60%	*thi*-1 - *ster*........75%

The above recombination frequencies for three four-point crosses with different outside markers, indicate that on the basis of a map location of *ster* between *leu*-3 and *mt*, abnormally high recombination frequencies are scored between *ster* and *leu*-3 and between *ster* and *mt*. According to Perkins *et al.*,(1959) the map distance between *mt* and *leu*-3 amounts to 16 map units. From the four-point cross analyses of these four-point crosses, it becomes evident that the number of double crossovers in this region exceeds that of singles. Other distances (*leu*-3 to *fr*, *mt* to *cr*

and, *mt* to *thi*-1 conform to the expected, if the map does not involve the intercept *mt* to *leu*-3. For example, the distance between *mt* and *cr* amounts to 17% and is therefore close to the expected 24 map units as given by Perkins *et al.*,1959. The calculated distance between *cr* and *leu*-3 on the other hand, is much too long (44 m.u. + 43 m.u. + 17 m.u. = 104 m.u.). Consequently, if the male sterility gene (*ster*) is located in Linkage Group I (as seems to be evident from the data obtained from two-point crosses with the exception of mutant 8455 *A*, *leu*-3, *ster*), it must be placed between *mt* and *leu*-3. The presence of *ster* in the genome causes an abnormallly high crossover frequency in the region of its location on Linkage Group I. Under these circumstances the results constitute an example of recombination enhancement of markers in the intercept *leu*-3 to *mt* by the mere presence of *ster* in that region.

An alternative explanation of the segregation data necessarily has to assume that the observed linkage between *ster* and *mt* is a quasi one and that consequently *ster* and *mt* are located on different chromosomes. Assuming quasi linkage an explanation for the predominance of parental types among the progeny of an *A*, *ster* x *a*, $ster^+$ cross has to be afforded. Such an explanation should necessarily rest on the assumption that certain genotypes are either preferentially produced (i.e.,parental types) or that selection against recombinant types takes place. Since the segregation data indicate an one-to-one ratio for reciprocal recombinant phenotypes (*A*, $ster^+$ and *a*, *ster*) partial elemination of these genotypes most likely occurred in the Non-Parental Ditype (NPD). In this respect it is interesting to note that tetrad analysis of the cross 8553 *A*, *leu*-3, *ster* x St.Lawrence *a* (Vigfusson,1969) yielded 4 Parental Ditypes (PD) 0 Non-Parental Ditypes (NPD) and 8 Tetratypes (TT). Hence a complete absence of NPD's. In the cross 10710-1 *A*, *leu*-3, *ster* x L *a*, *fl*, as reported in this

thesis 2 PD's, 0 NPD's and 4 TT's were obtained (Table VII).

Vigfusson (1969) found that male sterility is induced with greater ease in the genome of mating type *A* than in the genome of mating type *a* and hence predominance of parental types (*A*, *ster* and *a*, *ster*$^+$) in the progenies of the above mentioned crosses may reflect an increased stability of the genome rather than a preferential affinity of *ster* for mating type *A*.

Assuming quasi linkage between *ster* and *mt*, the following recombination frequencies can be calculated for the crosses

8553 *A*, *leu*-3, *ster* x B 110 *a*, *fl*.
8553 *A*, *leu*-3, *ster* x R2360 *a*, *cr*
8553 *A*, *leu*-3, *ster* x 56501 *a*, *thi*-1 :

outside marker *fr*	outside marker *cr*	outside marker *thi*-1
leu-3 - *mt*....20%(14%)*	*leu*-3 - *mt*....13%(14%)*	*leu*-3 - *mt*.....17%(14%)*
mt - *fr*.......38%(52%)*	*mt* - *cr*.......17%(18%)*	*mt* - *thi*-1.....22%(34%)*
fr - *leu*-3....18%(38%)*	*cr* - *leu*-3....30%(32%)*	*thi*-1 - *leu*-3..50%(48%)*

Although the above data deviate somewhat from the expected values, this deviation falls well within the variation as quoted in relevant literature:

leu-3 - *mt* ....5 m.u. (Barratt *et al.*,1954)
15 m.u. (Perkins *et al.*,1959)
14 m.u. (Perkins *et al.*,1962)

fr - *leu*-3.............27 m.u. (Perkins *et al.*,1959)
37 m.u. (Perkins *et al.*,1962)

mt - *cr*.................12 m.u. (Barratt *et al.*,1954)
27 m.u. (Perkins *et al.*,1959)
24 m.u. (Perkins *et al.*,1962)

mt - *thi*-1.............37 m.u. (Barratt *et al.*,1954)
34 m.u. (Perkins *et al.*,1959)
33 m.u. (Perkins *et al.*,1962)

---

*) expected recombination frequencies according to Perkins *et al.*,1959, in brackets.

Several crosses, *viz.*, 16009 *A*, *pan*-1, *ster* x 1633 *a*, *pab*-1
16009 *A*, *pan*-1, *ster* x 4894 *a*, *met*-7
16009 *A*, *pan*-1, *ster* x Y-153-M7 *a*, *ad*-8
5538 *A*, *pan*-1, *ster* x R2360 *a*, *cr*
5538 *A*, *pan*-1, *ster* x L *a*, *fl*
7232 *A*, *leu*-3, *ster* x 553 *a*, *pan*-1
involving pantothenic acid requiring strains in matings with non-requiring pantothenic acid strains, did not yield expected pantothenic acid progenies when the gene for male sterility(*ster*) was also involved in the cross. In one case (10299 *A*, *leu*-3, *ster* x 553 *a*, *pan*-1 no viable ascospores were obtained, although spore production appeared to be normal. In subsequent crosses (4) identical results were obtained.

Although no explanation for the above mentioned phenomena can be offered, it is evident that the genetic transmission of the pantothenic acid requirement is interfered with when the male sterility (*ster*) marker is incorporated into the genome of the opposite parent. In all the crosses, except for cross 10299 *A*, *leu*-3, *ster* x 553 *a*, *pan*-1 for which no data are available, the recombination frequencies for markers other than *pan*-1 were not affected by the absence of *pan*-1 segregants. Consequently, either meiotic repair of the *pan*-1 lesion occurred in the above crosses or, selection for *pan*-1^+ segregants took place. In cross 7232 *A*, *leu*-3, *ster* x 553 *a*, *pan*-1 the ascospore germination percent amounted to 43%, whereas in other crosses involving 7232 *A*, *leu*-3, *ster* germination percentages of 87-99% were scored. This difference in germination percentages seem to underline the possibility that selection of *pan*-1^+ segregants caused the above mentioned aberrant segregation.

Bibliography

Backus, M.P. 1939. The mechanics of conidial fertilization in Neurospora sitophila. Bull. Torrey Bot. Club 66:63-76.

Barratt, R.W., and W.N. Ogata. 1962. Neurospora Stock List. In: Neurospora Information Conference. N.A.S.-N.R.C. publ. 950:19-94.

Barratt, R.W., and W.N. Ogata. 1968. Neurospora Stock List. Fourth revision (June,1968). Neurospora Newsletter 13:25-95.

Barratt, R.W., D. Newmeyer, D.D. Perkins and L. Garnjobst. 1954. Map construction in Neurospora crassa. Advances in Genetics, Vol. 6.

Bistis, G.N., and J.R. Raper. 1963. Heterothallism and sexuality in Ascobolus stercorarius. Amer. Jour. Bot. 50:880-891.

Burnett, J.H. 1956. The mating system in fungi. I. New Phytologist 55:50-90.

Dodge, B.O. 1935. The mechanism of sexual reproduction in Neurospora. Mycologia 27:418-438.

Lewis, D. 1954. Comparative incompatibility in Angiosperms and Fungi. Adv. Genetics 6:235-285.

Lindegren, C.C. 1934a. The genetics of Neurospora. V. Self-sterile bisexual heterokaryons. Jour. Genet. 28:425-435.

Lindegren, C.C 1934b. The genetics of Neurospora. VI. Bisexual and akaryotic ascospores from N.crassa. Genetica 16:315-320.

Lindegren, C.C., V. Beanfield and R. Barber. 1939. Increasing the fertility of Neurospora by selective inbreeding. Bot. Gaz. 100:592-599.

Moreau, F., and C.Moruzi. 1932. Sur quelques aspects remarquables des cultures dispermes des Ascomycetes du genre Neurospora. Comt. Rend. Soc. Biol. III: 864-865.

Newmeyer, D. 1965. A pericentric inversion in Neurospora crassa. (Abstr.). Genetics 52:462-463.

Olive, L.S. 1958. On the evolution of heterothallism in fungi. Amer. Nat. 92:233-251.

Raper, J.R. 1960. The control of sex in fungi. Amer. Jour. Bot.47:794-808.

Smith, B.R. 1962. Mating type tests using a plating technique. Neurospora Newsletter 1:14.

Vigfusson, N.V. 1969. Sterility mutants in Neurospora crassa. Ph.D. Thesis, University of Alberta.

Vogel, H.J. 1956. A convenient growth medium for Neurospora. Microb. Gen. Bull.13:42-43.

Weijer, J. 1954. A genetical investigation into the td locus of Neurospora crassa. Genetica 28:173-252.

Weijer, J. , and J.Yang. 1966. Third division segregation for sexuality at the mating-type locus of Neurospora and its genetic implications. Can. J. Genet. Cyt. 8:807-817.

Westergaard, M., and H.K. Mitchell. 1947. Neurospora . V. A synthetic medium favoring sexual reproduction. Amer. Jour. Bot. 34:573-577.

Wheeler, H.E. 1954. Genetics and evolution of heterothallism in Glomerella. Phytopath. 44:342-345.

Wheeler, H.E., and J.W. McGahen. 1952. Genetics of Glomerella. X. Genes affecting sexual reproduction. Amer. Jour. Bot. 39:110-119.

Whitehouse, H.L.K. 1949. Heterothallism and sex in fungi. Biol.Rev. 24:411-447.

APPENDIX I

In order to demonstrate genetic segregation of male sterility (*ster*), Vigfusson (1969) crossed the *ster* mutants with a wild type (with respect to fertility) strain (St.Lawrence, *pan*-1, or *leu*-3). Results are shown in Table A along with ascospore germination percentages and recombination frequencies between *ster* and *mt*. Relative frequencies of *a* *vs*. *A* and *ster* *vs*. *ster*$^+$ are also given as an indication of the amount of selection taking place in single strand analysis. Vigfusson's data have been re-grouped in Table B in such a manner that reference is made to only those mutants which were employed in this thesis. Strain 8455 *A*, *leu*-3, *ster* has been excluded from the calculations of the percent recombination between *ster* and *mt* (*A* *vs*. *a*) since there is little or no selection against certain genotypes in single strand analysis of the offspring of mutant 8455 *A*, *leu*-3, *ster*.

Vigfusson determined segregation ratios between *ster* and *mt* for some of the mutants which have also been analysed in this thesis. His data are given in Table C.

The mutants used in the present study all represent so-called 'early mutants' with an early block in the sexual cycle. From his complementation data (Table D) it appears that 4 mutant genes are responsible for the early blocks. Strain 5366, 8455, and 8553 represent, respectively, 3 of these genes while strains 7232, 10299, 10710, 10718, 5538, and 16009 represent the fourth gene. The intergenic recombination data are compatible with this classification.

Table A Segregation of male sterility (st^-) and mating type in random spores from crosses of $ster^+$ a x ster x A

Mutant number	Percent ascospore germination	Number of spores analyzed	Segregation of sterility and mating type				Relative frequency of		Percent recombination between male sterility (ster) and mating type
			a $ster^+$	a ster	A $ster^+$	A ster	a : A	ster : $ster^+$	
5366	-	21 tetrads	15 PD:6 TT:0 NPD				-	-	-
7232	50.0	199	61	27	40	71	88 : 111	98 : 101	33.6
7384	not tested								
8455	54.6	365	114	72	90	89	186 : 179	161 : 204	45.0
8553	74.5	511	206	63	57	185	269 : 242	248 : 263	23.5
10299	55.0	220	84	36	35	65	120 : 100	101 : 119	32.2
10710	51.3	309	91	66	67	85	157 : 152	151 : 158	43.0
10718	86.0	315	103	55	54	103	158 : 157	158 : 157	31.6
5538	80.0	171	51	32	28	60	83 : 88	92 : 79	35.0
5926	not tested								
16009	75.5	169	65	28	20	56	93 : 76	84 : 85	27.8
7065	62.0	124	58	0	66	0	-	-	-
9312	73.0	146	77	0	70	0	-	-	-
10233	19.0	56	33	0	23	0	-	-	-
10589	69.0	69	30	0	39	0	-	-	-
10734	not tested								
7341	not tested								
9840	48.0	158	85	0	73	0	-	-	-
10402	49.0	162	82	0	80	0	-	-	-
10528	54.2	163	88	0	75	0	-	-	-
10979	89.0	178	86	0	92	0	-	-	-
10982	31.7	255	140	57	27	31	197 : 58	88 : 167	33.0
11042	58.0	174	87	0	87	0	-	-	-
16044	67.0	125	33	27	32	33	60 : 65	60 : 65	47.3

Table A - continued

Mutant number	Percent ascospore germination	Number of spores analyzed	Segregation of sterility and mating type				Relative frequency of		Percent recombination between male sterility and mating type
			a ster$^+$	a ster	A ster$^+$	A ster	a : A	ster : ster$^+$	
P-B-13-1	34.0	102	61	4	15	22	65 : 37	26 : 76	18.6
P-D-11-1	67.0	67	35	0	32	0	-	-	-
P-D-12-1	52.5	62	28	5	24	5	33 : 29	10 : 52	47.0
9961	72.0	90	40	0	50	0	-	-	-
10777	67.0	82	41	0	41	0	-	-	-
12042	17.5	94	18	0	76	0	-	-	-
12365	67.8	252	128	0	124	0	-	-	-
15218	51.5	181	47	0	134	0	-	-	-

Table B

Strain Number	Percent Germination	Number of spores	Segregation				Relative Frequency of		Percent Recombination		
			A ster$^+$	a ster	A ster	ster$^+$ a ster	a/A	ster/ster$^+$	Total	a	A
7232	50	199	61	27	40	71	88/111	98/101	33.6	30.7	36.0
8455	54	365	114	72	90	89	186/179	161/204	(45.0)	(38.8)	(50.2)
8553	74	511	206	63	57	185	269/242	248/263	23.5	23.4	23.6
10299	55	220	84	36	35	65	120/100	101/119	32.2	30.0	35.0
10710	51	309	91	66	67	85	151/152	151/153	43.0	42.0	44.1
10718	86	315	103	55	54	103	158/157	158/157	31.6	34.8	34.4
5538	80	171	51	32	28	60	83/88	92/79	35.0	38.6	31.8
16009	75	169	65	28	20	56	93/76	84/85	27.3	30.1	26.3
								Average (excl.8455)	35.1	32.8	33.1

TABLE C ANALYSIS OF RANDOM ASCOSPORES FROM RECIPROCAL CROSSES OF SOME MALE STERILE MUTANTS WITH WILD TYPE ST. LAWRENCE

Mutant crossed and direction of cross*	Number of spores analyzed	Segregation of mating type and sterility			
		a ster$^+$	a ster	A ster$^+$	A ster
wt. x 9312	89	49	0	40	0
9312 x wt.	57	28	0	29	0
wt. x 9840	26	15	0	11	0
9840 x wt.	132	70	0	62	0
wt. x 10402	82	40	0	42	0
10402 x wt.	80	42	0	38	0
wt. x 10982	55	28	19	3	5
10982 x wt.	42	29	5	5	3
wt. x P-B-13-1	55	30	2	10	13
P-B-13-1 x wt.	30	22	2	1	5
wt. x 8455	168	46	39	37	46
8455 x wt.	89	19	29	14	27
wt. x 8553	100	36	10	13	41
8533 x wt.	128	56	14	15	43
wt. x 10299	99	38	18	19	24
10299 x wt.	121	46	18	16	41
wt. x 10718	88	26	9	18	35
10718 x wt.	92	35	18	9	30
wt. x 5538	71	21	17	11	22
5538 x wt.	100	30	15	17	38
wt. x 16009	69	30	12	6	21
16009 x wt.	100	35	16	14	35

* The first parent listed is the protoperithecial parent.

TABLE D OCCURRENCE OF RECOMBINATION IN DIFFERENT STER CROSSES

	8455	8553	10299	10710	10718	5366	5538	16009
7232	+	+	0	0	0	+	-	-
	8455	+	+	+	+	0	+	+
		8553	+	+	+	+	+	+
			10299	0	-	0	-	0
				10710	0	+	-	0
					10718	0	-	-
						5366	0	+
							5538	0

0 = No offspring available

+ = Recombinants isolated

- = No recombinants

B29943